Critical Role of cRel Subunit of NF- κ B in Sepsis Survival[†]

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NF- κ B is a critical regulator of gene expression during severe infections. NF- κ B comprises homo- and heterodimers of proteins from the Rel family. Among them, p50 and p65 have been clearly implicated in the pathophysiology of sepsis. In contrast, the role of cRel in sepsis is still controversial and has been poorly studied in single-pathogen infections. We aimed to investigate the consequences of cRel deficiency in a cecal ligation and puncture (CLP) model of sepsis. We have approached the underlying mechanisms of host defense by analyzing bacterial clearance, systemic inflammation, and the distribution of spleen dendritic cell subsets. Moreover, by using a genome-wide technology, we have also analyzed the CLP-induced modifications in gene expression profiles both in wild-type (wt) and in *rel*^{-/-} mice. The absence of cRel enhances mortality due to polymicrobial sepsis. Despite normal pathogen clearance, cRel deficiency leads to an altered systemic inflammatory response associated with a sustained loss of the spleen lymphoid dendritic cells. Furthermore, a whole-blood microarray study reveals that the differential outcome between wt and *rel*^{-/-} mice during sepsis is preceded by remarkable changes in the expression of hundreds of genes involved in aspects of host-pathogen interaction, such as host survival and lipid metabolism. In conclusion, cRel is a key NF- κ B member required for host antimicrobial defenses and a regulatory transcription subunit that controls the inflammatory and immune responses in severe infection.

Severe sepsis is a complex syndrome characterized by an amplified host inflammatory response leading to organ dysfunction. Host-pathogen interactions mediated through Toll-like receptors (TLRs) stimulate the production of proinflammatory cytokines, chemokines, and adhesion and immune activation molecules (23, 35). This proinflammatory response is followed by a compensatory immunosuppressive response that combines various quantitative and functional defects of immune cells (17). Pathogen-induced cellular modifications are accompanied by marked changes in host gene expression (29), with NF- κ B transcription factors playing a pivotal role in this modulation.

During severe infections, NF- κ B is crucial for the regulation of immune/inflammatory responses and the control of cell proliferation/apoptosis (2, 3, 27). NF- κ B transcription factors consist of homo- and heterodimers of the Rel protein family; p65

(RelA), RelB, cRel, p50, p52, p65, RelB, and cRel are transcriptionally active members of the NF- κ B family, whereas p50 and p52 serve primarily as nontransactivating DNA binding subunits (13). The major importance of the p50 and p65 subunits in the pathophysiology of sepsis has been highlighted in both animal models and human pathology (6). In contrast, the role and the significance of cRel during infectious challenges remain poorly defined.

Mice deficient for the cRel subunit (*rel*^{-/-} mice) exhibit compromised immune function and a variable response to individual pathogen infections (12, 25). Despite the fact that the immune system in the main develops normally in *rel*^{-/-} mice, these animals display defects in lymphocyte proliferation and humoral immunity (5, 7, 25). In animal models of sepsis, *rel*^{-/-} mice show normal resistance to *Trichuris muris* (1) and lymphocytic choriomeningitis virus (9). In contrast, they are susceptible to *Leishmania major* (14), *Toxoplasma gondii* (28), influenza virus (15), and *Listeria monocytogenes* (9). Despite these reports, the contribution of cRel to innate host responses directed toward common bacterial infections has not been studied, and the potential function of this NF- κ B member in the defense against acute bacterial infection has yet to be investigated *in vivo*.

In this report, we have investigated the consequences that

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cRel subunit deficiency has for survival and the host systemic inflammatory response in mice subjected to a model of polymicrobial peritonitis.

MATERIALS AND METHODS

Mice. Wild-type (wt) C57BL/6J female mice, 8 to 12 weeks old, were purchased from Charles River Laboratories. *rel*^{-/-} mice were kindly provided by S. Gerondakis (The Burnet Institute, Melbourne, Australia). Experiments were conducted in accordance with guidelines of the Cochin Institute, in compliance with the European animal welfare regulation.

Abs and reagents. The following antibodies (Abs) were purchased: anti-p65, anti-cRel, or anti-p50 polyclonal Abs (Santa Cruz Biotechnology); anti-CD11c magnetic beads and CD11c, CD8 α , CD11b, and anti-CD16/CD32 Fc block fluorescent antibodies (Miltenyi Biotec); and horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG and goat anti-mouse IgG (Pierce). Tienam (imipenem-cilastatin; used at 25 mg/kg in 0.5 ml saline) (Merck Sharp & Dohme), lipopolysaccharide (LPS) (*Escherichia coli* K-12 ultrapure LPS; Invivogen). SDS, glycerol orthovanadate, sodium pyrophosphate, and β -glycerophosphate were purchased from Sigma. Nonidet P-40 and Complete proteinase inhibitor cocktail tablets 25X were from Roche. Sepharose beads were streptavidin high performance from GE Healthcare. ECL Plus Western blotting detection reagents were purchased from Amersham and X-ray film from Pierce (CL-XPosure film; Pierce). The NF- κ B consensus probes used for electrophoretic mobility shift assay (EMSA) and DNA pull-down assay were provided by Promega.

Model of polymicrobial sepsis. We used a sublethal model of cecal ligation and puncture (CLP). Briefly, mice were anesthetized by an intraperitoneal injection of ketamine and xylazine. After a midline incision (<1 cm), the cecum was exposed, ligatured at its external third, and punctured through and through with a 21-gauge needle. The incision was sutured in layers, and animals were resuscitated with an intraperitoneal injection of 1 ml saline. Controls were sham-operated mice undergoing abdominal surgery, but with only exposition of cecum without CLP. Six hours following surgery and then every 12 h for 3 days, mice received an intraperitoneal injection of antibiotics (imipenem-cilastatin [Tienam; Merck Sharp & Dohme]; 25 mg/kg in 0.5 ml saline).

Assessment of bacterial dissemination. At different time points after surgery, mice were humanely killed, and blood samples of 0.5 to 1.0 ml were obtained by cardiac puncture. Bacterial dissemination was indirectly assessed through spleen, liver, and blood bacterial cultures to quantify the number of CFU. Spleen and liver were removed and mechanically homogenized under sterile conditions. Blood, spleen, and liver homogenates were subjected to serial 10-fold dilutions. Bacteria were quantified in tryptic soy agar (for spleen homogenates) or in Todd-Hewitt (TH) agar (for blood) after 24 h.

Determination of cytokines levels in mouse sera. Plasma interleukin-1 β (IL-1 β), IL-6, IL-12, gamma interferon (IFN- γ), tumor necrosis factor alpha (TNF- α), and IL-10 concentrations were quantified using quantitative multiplex assays (Bio-Plex cytokines; Bio-Rad) according to the manufacturer's instructions. Duplicates and means were obtained from 3 to 13 mice.

Preparation of DCs. Bone marrow-derived dendritic cells (BMDCs) were made from BM cells flushed from femurs and tibias (21). Cells were seeded in 24-well plates at the concentration of 1×10^6 /ml in RPMI medium supplemented with 5% fetal calf serum (FCS) and antibiotics in the presence of supernatant (4% vol/vol) from J558 cells transduced with murine granulocyte-macrophage colony-stimulating factor (GM-CSF). Cells were cultured for 6 days, with medium replacement at days 3 and 6. Spleen dendritic cells were prepared from collagenase-digested spleens. Cell suspensions were incubated with anti-CD11c magnetic beads to collect purified CD11c⁺ DCs (magnetic-activated cell separation CD11c isolation kit; Miltenyi Biotec). The purity of the positive fraction was generally >80% as confirmed by CD11c staining. Purified CD11c⁺ DCs were then stained with fluorescence-coupled CD11c and CD8 α antibodies to analyze the distribution of subsets by flow cytometry.

Cell stimulation and nuclear extract preparation. Briefly, BMDCs were left untreated or stimulated with LPS (1 μ g/ml) for 2 h. The cytosolic fraction was extracted in buffer A (10 mM HEPES-NaOH [pH 7.6], 3 mM MgCl₂, 10 mM KCl, 5% glycerol, 0.1% Nonidet P-40) containing 1 mM vanadate, 10 mM NaF, 1 mM sodium pyrophosphate, 25 mM β -glycerophosphate, and Complete proteinase inhibitor cocktail tablets 25X. Cells were incubated for 10 min at +4°C and centrifuged at 10,000 rpm for 2 min. Nuclear proteins were obtained by treatment of the pellet with buffer B (1 mM vanadate, 10 mM NaF, 1 mM sodium pyrophosphate, 25 mM β -glycerophosphate, and 300 mM KCl) and centrifuged after 30 min at +4°C and 15,000 rpm for 20 min. Finally, the nuclear fraction was diluted in buffer A.

EMSA and DNA pull-down assay. EMSA and DNA pull-down assay were used to analyze the NF- κ B DNA binding activity as previously described (11, 24). The probes used were nfkb wt (5'-AGTTGAGGGGACTTCCAGGC-3' and 5'-GCCTGGGAAAGTCCCTCAACT-3') and nfkb mut (5'-AGTTGAGGCGA CTTTCCAGGC-3' and 5'-GCCTGGGAAAGTCGCTCAACT-3'). For supershift assays in EMSA, 1 μ g of anti-RelA, anti-cRel, or anti-p50 polyclonal Ab was added to the reaction mixture. For DNA pull-down experiments, nuclear extracts were incubated with 1 μ g of double-strand consensus or control 5'-biotinylated probe for 1 h at +4°C. Twenty-five microliters of Sepharose beads was used to capture the biotinylated probe (15 min at +4°C). Proteins associated with the probe were eluted at 95°C for 5 min in Laemmli Buffer (Bio-Rad).

Western Blot analysis. Samples were loaded onto 10% denatured polyacrylamide gels with 1 $\times$ Tris-glycine (TG)-1% SDS. The migrated proteins were transferred to nitrocellulose membrane (38). The membrane was incubated in blocking buffer (1 $\times$ Tris-buffered saline [TBS] and 0.1% Tween 20 with 5% nonfat dry milk) for 1 h at room temperature. Incubation with primary Ab overnight at 4°C was followed by incubation with HRP-conjugated secondary Ab for 1 h at room temperature. Proteins were detected by addition of ECL Plus and exposure to X-ray film.

Microarray analysis. Whole-blood samples were collected at day 1 after sepsis induction from sham-operated and CLP-treated *rel*^{-/-} and wt mice. RNA extraction was performed with a TRIzol Plus RNA purification kit (Invitrogen) according to the manufacturer's protocol and analyzed using an Agilent 2100 Bioanalyzer (Agilent Technologies). RNA was used to make biotin-labeled cRNA using the SuperScript double-stranded cDNA synthesis kit (Invitrogen) and BioArray high-yield RNA transcript labeling kit (Enzo). Biotin-labeled cRNA was fragmented before being hybridized to the GeneChip mouse gene 1.0 ST array (Affymetrix), which contains 28,944 murine genes and expressed sequence tags (ESTs). The arrays were analyzed with a confocal scanner and with the MicroArray Suite 5.0 Gene Expression analysis program (Affymetrix). Fluorescence data were imported into two analysis software programs, Affymetrix Expression Console and R Bioconductor (<http://www.bioconductor.org>). Gene expression levels were calculated using the RMA algorithm in Expression Console, and flags were computed using a custom algorithm within. To limit potentially biased measurement (background or saturating), all probes for which normalized intensity measures were outside a confidence interval were flagged 0. The confidence interval was a 2-fold standard deviation from the mean intensity of each microarray. Three probe lists were used for each comparison according to flagged measurement in the relevant chips. The "PP" list is made of probes flagged only as "Present" for all microarrays involved in the comparison. The "P50%" list was created by filtering probes flagged as "Present" for at least half of the chips. The "All" list is made of all probes without any filter. Data were subsequently subjected to Ingenuity pathway analysis (IPA) (Ingenuity Systems, Inc., Redwood City, CA) to model relationships among genes and proteins and to construct putative pathways and relevant biological processes.

Statistical analysis. Survival curves were analyzed using the Kaplan-Meier method and compared using the log rank test. Continuous variables were expressed as median and interquartile range and displayed using box plots or scatter plots. They were represented as mean $\pm$ standard deviation (SD) and compared using the Student *t* test. Categorical variables were compared using the Fisher exact test. *P* values lower than 0.05 indicated statistically significant differences. For microarray analysis, independent samples were compared by computing fold ratios and filtered at 2-fold. Cluster analysis was performed by hierarchical clustering, using the Spearman correlation similarity measure and average linkage algorithm.

Microarray data accession number. The microarray data have been deposited into ArrayExpress under accession number E-MEXP-2944.

RESULTS

LPS activates the p50, p65, and cRel subunits of NF- κ B. To study the involvement of cRel in antimicrobial immune cell responses, the subunit composition of κ B binding complexes was first analyzed in LPS-stimulated BMDCs. As shown in Fig. 1A, NF- κ B DNA binding activity measured by EMSA was strongly induced in BMDCs upon 2 h of stimulation with LPS. The abundance of the κ B DNA binding complexes dramatically decreased with the addition of antisera against cRel, p65, and p50, demonstrating the presence of these three subunits, including cRel, within the LPS-induced NF- κ B complexes (Fig.

FIG. 1. NF- κ B/Rel subunit composition in dendritic cells upon LPS stimulation. BMDCs were left untreated (phosphate-buffered saline [PBS]) or stimulated for 2 h with LPS. Nuclear extracts were prepared and analyzed by EMSA (A) and DNA pull-down assay (B) for binding to the consensus κ B site. Nuclear extracts were also preincubated with anti-p65, anti-p50, and anti-cRel antibodies prior to incubation with the κ B site oligonucleotide probe. The arrows correspond to the supershifted bands with anti-cRel and anti-p50 antibodies (A).

1A). Further evidence for the presence of cRel and p50 in these complexes was the presence of specific cRel and p50 bands in the supershift experiment. These results were validated by DNA pull-down assay, illustrated in Fig. 1B. Together, these results indicated that besides the activation of the prototypic p65 and p50 proteins, LPS-activated NF- κ B complexes include cRel.

Sublethal polymicrobial sepsis induces increased mortality in $rel^{-/-}$ mice. In order to investigate the importance of cRel in the host response to acute infection, survival of wild-type (wt) and $rel^{-/-}$ mice was evaluated using a model of sublethal polymicrobial sepsis. As shown in Fig. 2, the incidence of mortality was dramatically and significantly increased in $rel^{-/-}$ mice (12/20; 60%) compared to wt animals (4/18; 22%) ($P < 0.05$), suggesting for the first time the critical role of the cRel subunit in survival of polymicrobial sepsis.

Absence of cRel and bacterial clearance. As bacterial clearance is essential for survival of infection, the consequences of

a lack of *rel* on systemic bacterial dissemination were investigated. As illustrated in Fig. 3, wt and *rel*-deficient mice exhibited similar bacterial counts in liver, spleen, and blood, indicating that the increased mortality seen in $rel^{-/-}$ mice was not related to impaired pathogen clearance.

cRel deficiency leads to an altered inflammatory response. An imbalance of pro- and anti-inflammatory cytokine responses has been linked to the risk of death in both experimental and clinical sepsis models. Hence, we measured plasma levels of several cytokines at days 1, 3, 5, and 7 after CLP. In wt mice, induction of sepsis led to a moderate release of pro-inflammatory cytokines at day 1, followed by a substantial increase at days 3 and 5 for TNF- α , IL-1 β , and IFN- γ (Fig. 4). Both IL-6 and IL-10 levels remained unchanged during the sepsis course. Compared to wt mice, in the early phase, *rel*-deficient mice had a significantly enhanced release of pro-inflammatory cytokines (TNF- α , IL-1 β , IL-6, IL-12p70, and IFN- γ). In contrast, at day 7, $rel^{-/-}$ mice exhibited a significant lower plasma level of IL-1 β .

Absence of cRel leads to sustained depletion of spleen dendritic cells upon polymicrobial sepsis. DCs link innate immunity and adaptive immunity and are critical in mounting and coordinating host immune responses against infection. Moreover, the importance of DCs in both the early and late phases of sepsis has been established (4, 31), with these cells having been reported to be required for survival of polymicrobial sepsis (37). In wt mice, a selective depletion of the spleen lymphoid DC subset CD11c⁺ CD8 α ⁺ was found at day 1, followed by a full replenishment at day 7 after CLP. Although delayed, sustained spleen DC depletion was observed in *rel*-deficient mice from day 3 until day 14 after surgery (Fig. 5 and data not shown). Recovery time in $rel^{-/-}$ mice was not established.

Genome-wide analysis of the host systemic response to polymicrobial sepsis. To further explore the increased mortality rate in the $rel^{-/-}$ animals, blood from both wt and *rel*-deficient mice was subjected to global gene expression analysis at day 1. First, microarray analysis did not reveal major differences in the global gene expression in sham-operated wt and $rel^{-/-}$ mice (data not

FIG. 2. Survival rates of $rel^{-/-}$ and wild-type animals with polymicrobial sepsis. Sublethal polymicrobial sepsis was induced by CLP in wild-type and $rel^{-/-}$ mice. Survival of wt ($n = 18$) or $rel^{-/-}$ ($n = 20$) mice was monitored for 12 days (log rank test, $P < 0.05$).

FIG. 3. Assessment of bacterial load in mice upon induction of polymicrobial sepsis. The bacterial counts in wt (black circles) and *rel*^{-/-} (white circles) mice were determined at days 1, 3, 5, and 7 after CLP. Bacterial dissemination was assessed through quantitative liver, spleen, and blood bacterial cultures. Bacterial counts were compared by Student's two-tailed *t* test.

shown). CLP significantly altered the expression of 3,414 probe sets in wt mice and 3,169 in *rel*^{-/-} mice (Fig. 6). Among those, the expression of 1,897 probe sets was modified in both groups of mice and corresponded to the core response (1,051 versus 1,026 upregulated and 846 versus 871 downregulated in the *rel*^{-/-} and wt mice, respectively). Heat map analysis indicated that these common probe sets consisted of genes involved mainly with the inflammatory response for the upregulated core response and cellular development for the downregulated common probe sets (see Tables S1 and S2 in the supplemental material). Expression of 1,517 probe sets was specifically modified in wt mice (Fig. 6). Functional grouping of these probe sets showed that organism survival and the cell cycle were the main functions for the up- and downregulated genes, respectively (see Tables S3 and S4 in the supplemental material). Finally, 1,272 probe sets were specifically modulated in the absence of cRel. Their corresponding functions concerned lipid metabolism for the upregulated genes and amino acid metabolism for the downregulated ones (see Tables S5 and S6 in the supplemental material).

DISCUSSION

Using a sublethal polymicrobial model of sepsis, we report for the first time that cRel deficiency leads to an increased rate of mortality, an altered systemic host response with an early hyperinflammatory profile, and a sustained depletion of spleen lymphoid DCs. Furthermore, we show that the absence of cRel leads to a dramatic modification of CLP-induced gene expression.

The phenotypic characteristics of *rel*^{-/-} mice observed in this model of polymicrobial sepsis could either result directly from the downregulation of cRel-dependent gene expression or be a consequence of altered NF-κB-dependent transcription arising from the replacement of cRel by other members of the Rel family in the activated NF-κB dimers. Few studies have analyzed the direct impact of cRel on gene expression. By combining genome-scale location and specific NF-κB subunit antibodies in U937 cells, Schreiber and coworkers have shown that cRel binds to 83 κB target genes following LPS stimula-

FIG. 4. Analysis of the systemic cytokine response in mouse sera upon induction of polymicrobial sepsis. Polymicrobial sepsis was induced by CLP in wt and *rel*^{-/-} mice. Blood samples were collected at days 1, 3, 5, and 7 after CLP. The concentrations of IL-1β, IL-6, TNF-α, IL-12p40, IL-10, and IFN-γ were determined by Multiplex. Duplicates and means were obtained from 3 to 13 mice. *, *P* < 0.05; **, *P* < 0.01.

tion (36). Among these 83 genes, 12 genes require exclusively cRel binding for their expression. Notably, none of these 12 genes has a clear-cut role in the host response to infection. Moreover, only one of them, *Cyp51*, has been shown in our microarray analysis to be affected by the absence of cRel. Hence, compared to the case in wt mice, *Cyp51* was downregulated 2.2-fold in the *rel*^{-/-} mutants after CLP, confirming the role of cRel in the transcriptional activity of this gene. The specificity of NF-κB transcriptional activity is also highly dependent on the subunit composition of the potential dimers (30). In *rel*^{-/-} mice, cRel:cRel, cRel:p65, cRel:p50, and cRel:p52 dimers are absent and therefore might be compensated for by the remaining dimers, thereby modifying the immune host response to sepsis. For example, in *rel*^{-/-} macrophages, the p50:cRel heterodimer could be replaced by the p50:p65 heterodimer, a more powerful transcriptional activator (9). Because p50:p65 plays a predominant role in proinflammatory

gene transcription at the early phase of sepsis (13, 6, 41) the enhanced release of the proinflammatory cytokines observed at day 1 after CLP in the absence of cRel (Fig. 4) might be at least explained by such compensation (34). This exaggerated inflammation is usually considered a hallmark in the development of organ dysfunction leading to death during sepsis and might play a role in the increased mortality of our model (17, 40). Similarly, compensation for absence of cRel by p65 can explain the delayed depletion of spleen lymphoid DCs (Fig. 5), since the p50:p65 heterodimer has been reported to play an antiapoptotic role (3). Two mechanisms may be involved in the temporary effect of such compensation: (i) the secondary compensation of cRel by RelB that antagonizes p65 (30) and (ii) the dysfunction of p65 that occurs 2 days after the beginning of sepsis (6), limiting the protective effect initially observed.

In vitro, the importance of cRel in the control of the adaptive immunity has been well documented. However, the potential

FIG. 5. Distribution of the CD11c⁺ CD8α⁺ spleen lymphoid DC subset upon induction of polymicrobial sepsis. Spleen dendritic cells were prepared from collagenase D-digested spleens harvested from wt and *rel*^{-/-} mice at days 1, 3, 5, and 7 after CLP. CD11c⁺ purified DCs (CD11c microbead separation kit) were labeled with anti-CD11c and anti-CD8α antibodies. The figure represents the ratio of CD11c⁺ CD8α⁺ for CLP-treated mice to CD11c⁺ CD8α⁺ for sham-treated mice. Statistical comparison was done by Student's two-tailed *t* test. *, *P* < 0.05; **, *P* < 0.01.

function of this NF-κB subunit in the innate immune response and in survival during polymicrobial infection has never been explored. *rel*^{-/-} mice have been previously reported to be susceptible to different single microbial infections (9, 14, 15, 28), but the role of cRel in antibacterial host defense mechanisms has been explored only in *Listeria monocytogenes* infection (9). However, in that model, only the pathogen clearance, which was defective in the mutant mice, was evaluated. In contrast, in our sublethal polymicrobial model of sepsis, we demonstrate an increased mortality rate in the *rel*^{-/-} group despite a normal bacterial clearance, even if we should be cautious in this statement, essentially at days 5 and 7, due to the limited number of surviving mice analyzed. This apparent discrepancy may be explained by the nature of the pathogen, which influences dramatically the antibacterial response (i.e., an intracellular pathogen for *Listeria monocytogenes* versus extracellular pathogens for the intestinal bacteria) (33).

The poor prognosis of *rel*-deficient mice may be related either to an overwhelming early systemic inflammatory response, as discussed above, or to a secondary immunosuppression. Indeed, at day 7 *rel*^{-/-} mice have a decreased release of IL-1β (Fig. 4) and a sustained depletion of spleen lymphoid DCs (Fig. 5). Potential prognostic value of the selective depletion of spleen DCs, which are essential to develop an efficient immune response (37), has been reported for patients with sepsis (20) and in animal models of sepsis (32). We have recently shown that TLR2 and TLR4 signaling is involved in the mechanisms leading to depletion of spleen DCs following polymicrobial sepsis (31). Analysis of the expression of proteins involved in TLR signaling in our microarray analysis reveals that only the factor Tollip is downregulated in *rel*^{-/-} mice after CLP (data not shown). Tollip is known to dampen TLR2- and TLR4-mediated signaling in mammals, suggesting that a lack of cRel might lead to a hyperactivation of the TLR signaling responsible for the increased DC depletion. Finally, as cRel plays an important role in the adaptive immune response, controlling the normal functions of B cells, T cells,

FIG. 6. CLP-regulated gene expression in mouse whole blood. Blood samples were collected from both sham-operated and CLP-subjected wt and *rel*^{-/-} mice. (A) Venn diagram of probes differentially expressed in wt and in *rel*^{-/-} mice after CLP versus sham laparotomy. Numbers in the overlapping region of the Venn diagram represent common regulated genes. In this area, dd, downregulated in both groups of mice; du, downregulated in wt and upregulated in *rel*^{-/-} mice; ud, upregulated in wt and downregulated in *rel*^{-/-} mice; uu, upregulated in both groups of mice. Numbers of wt-specific genes are shown in the left circle, and numbers of *rel*^{-/-}-specific genes are shown in the right circle. d, downregulated; u, upregulated. (B) Heat maps depicting the impact of CLP on the mRNA abundance of specific probe sets. Heat maps of the 3,414 probe sets were classified as wt-modified probe sets (*n* = 1,517, left panel), common probe sets (*n* = 1,897, middle panel), and *rel*^{-/-}-modified probe sets (*n* = 1,272, right panel). The probe list was subjected to heat mapping based on the values obtained from the whole-blood samples (columns). Probes were classified by similarity of expression profile among the samples. Colors indicate the normalized expression values below (green) and above (red) the median expression.

and antigen-presenting cells (APCs) (5, 7, 12, 25), we can hypothesize that *rel*^{-/-} mice are unable to develop the efficient adaptive immunity that is necessary to survive during CLP (16, 19, 22).

To further explore the underlying mechanisms leading to the increased mortality of *rel*^{-/-} mice, we have employed genome-wide microarray technology to analyze the CLP-induced gene expression in both *rel*^{-/-} and wt animals. Multiple studies have demonstrated that this strategy can identify new genes and unknown pathways involved in the anti-infectious host response (8, 26). As already reported, CLP produced dramatic changes in the probe set profile, modifying 11.8% of the whole genome (3,414/28,944) (10, 39). The difference in gene profiles

between wt and *rel*^{-/-} mice is probably a key to understanding the mechanisms responsible for the differential outcomes of sepsis. Ingenuity analysis of gene expression profiles highlighted a subgroup of 63 genes involved in "organism survival," which are upregulated in wt but not in *rel*^{-/-} animals (Fig. 6; see Table S3 in the supplemental material). Among those genes, *Bcl-2l 11*, a member of the Bcl2 family, is upregulated after CLP only in wt mice. This result is consistent with the deleterious role of apoptosis in sepsis outcome (18). In addition, gene ontology revealed potential regulation by cRel of new genes involved in previously identified pathways such as the cell cycle and amino acid metabolism. The discovery of these new genes and gene families has been made possible through our microarray study and may constitute the basis for additional experiments useful in providing new insights into the biology of cRel and its role in gene transcription. However, as the host response to sepsis is highly compartmentalized with tissue specificity of gene expression (9), our data should be considered only for the systemic response to CLP.

In conclusion, the present study highlights for the first time the critical role of cRel in the antimicrobial host defense. The identification of cRel target genes associated with inflammation and survival of sepsis might lead to potential NF- κ B-targeted immunomodulation in severe infection. However, the clinical relevance in humans has to be validated.

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